Bioinformatic Approaches to Detecting Copy Number Variation in Next Generation Sequencing Data for Clinical Diagnostics

Straver, R.

2018

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Chapter 4

Mosaic maternal 10qter deletions are associated with FRA10B expansions and may cause false positive NIPT results

Karin Huijsdens-van Amsterdam*, Roy Straver*, Merel C. van Maarle, Alida C. Knegt, Diane van Opstal, Frank Sleutels, Dominique Smeets, and Erik A. Sistermans

* Equal contributors

This chapter has been published in *Genetics in Medicine*.
2018 Mar 1;Epub. doi: 10.1038/gim.2018.32.

Abstract

Purpose: Using genome wide non-invasive prenatal testing (NIPT) we detected a 20 Mb specific deletion starting at 10q25 in eight pregnancies. The deletion could not be confirmed by invasive testing. Since all 10(q25→qter) deletions started close to the FRA10B fragile site in 10q25, we aimed at investigating whether the pregnant women indeed were carriers of FRA10B.
Methods: We performed NIPT analysis for all autosomes using single read sequencing. Analysis was done with the WISECONDOR algorithm. Culture of blood lymphocytes with bromodeoxyuridine (BrdU) was used to detect FRA10B expansions. FISH and array analysis were used to find maternal and/or fetal deletions.
Results: We confirmed the presence of a FRA10B expansion in 4 out of 4 tested mothers. FISH and array analysis confirmed the presence of a maternal mosaic deletion of 10(q25→qter).
Conclusions: The recurring 10(q25→qter) deletion detected with NIPT is a false positive result caused by a maternal low-level mosaic deletion, associated with FRA10B expansions. This has important consequences for clinical follow up, as invasive procedures are unnecessary. Expanded maternal FRA10B repeats should be added to the growing group of variants in the maternal genome that may cause false positive NIPT results.

Introduction

Non-invasive prenatal testing (NIPT) is now used worldwide for the detection of common aneuploidies. With NIPT, cell free fetal DNA (cffDNA) in maternal plasma is analyzed. Even though NIPT is a highly sensitive and specific screening test, false positive results are sometimes obtained. Most false positive results can be explained by confined placental mosaicism (CPM), since the primary source of fetal DNA in the maternal circulation is the placental cytotrophoblast [1]. Other causes for false positive NIPT results are maternal mosaicism [2], maternal CNVs [3], maternal cancer [4], or a vanishing twin [5].

We detected a ∼20 Mb deletion of 10(q25→qter) in eight independent samples tested. During extensive follow up investigations, the detected loss on chromosome 10 was initially not seen in fetal, maternal, nor placental tissue. The deletions showed strong similarities in their breakpoint locations, while sample analyses were performed in three different centers and at different points in time, thus ruling out run- and lab specific effects as a cause. In an effort to explain these false positive NIPT results, it was noticed that the proximal breakpoint of the deletion occurred at the location of FRA10B and we questioned whether this fragile site could be involved in the apparent NIPT deletion.

Fragile sites are heritable specific chromosome loci that exhibit an increased frequency of gaps, poor staining, constrictions or breaks when chromosomes are exposed to partial DNA replication inhibition. They are classified as common or rare (<5% of the population) and are further subdivided into different groups based on their specific induction chemistry [6]. There are two known fragile sites for 10q25; FRA10B and FRA10E. The

latter fragile site is a common fragile site and thus present in all (or nearly all) individuals. FRA10B however, is a rare and non-folate sensitive fragile site. Although it classifies as rare, the population frequency of cytogenetic expression of FRA10B still is ∼1/40 in the Australian population [7]. FRA10B contains AT-rich (91%) repeats. Its expression is associated with expansion of one or more repeats, showing an increase of up to several kilobases of DNA [8, 9]. The expansion of the AT-rich inverted repeats may generate perturbation of DNA replication [10]. Furthermore, the expanded inverted AT-rich repeats form hairpin structures that may contribute to their further expansion [9].

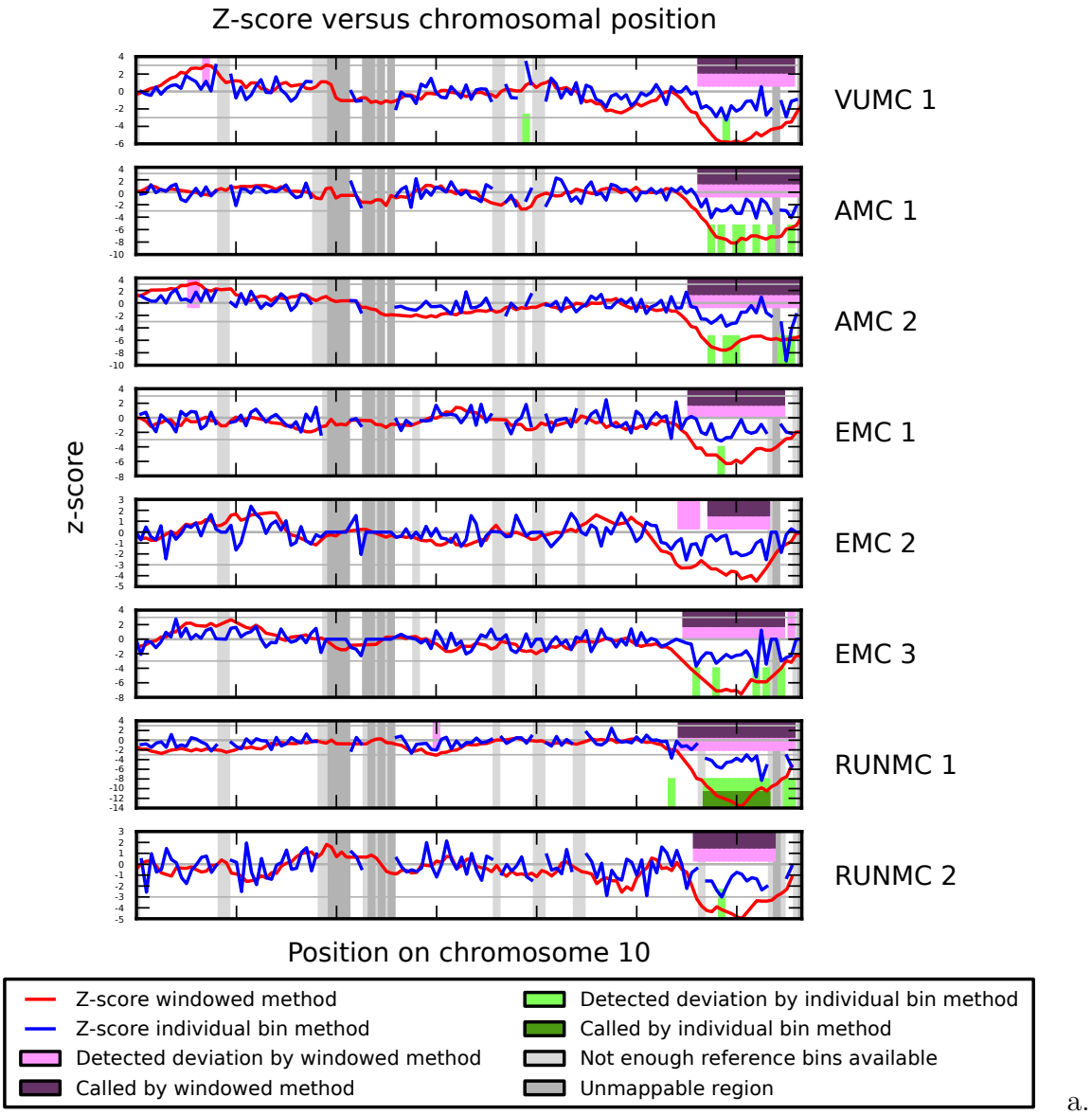
Materials and Methods

All samples were analysed as part of the Dutch TRIDENT study, which includes the analysis of aberrations other than trisomy 21, 18 or 13 [11]. Bioinformatic analysis was performed using WISECONDOR at default settings as described in [12]. Furthermore, adaptations to WISECONDOR were made to pinpoint the affected area more precisely. We increased the resolution by changing the bin size from 1 megabase to 250 kilobases. Speed and precision were improved by replacing LOESS GC-correction with Principal Component Analysis (PCA) where the PCA-transform was determined over the reference sample set. After mapping data to the PCA dimensions (using the first 3 components), the original data was reconstructed and the difference between this reconstruction and the actual signal was taken as the read depth input per bin for WISECONDOR. The windowed z-score approach was replaced by a segmentation algorithm that focusses on finding the strongest Stouffers z-scores over all possible windows per chromosome, allowing optimization of the call down to single bins. As these changes increase the amount of tests per sample, the z-score threshold for significant aberrations had to increase as well. Instead of the usual threshold of 3, the script determined the required z-score to be 4.8 for our purposes. The new script is available from https://github.com/VUmcCGP/wisecondor.

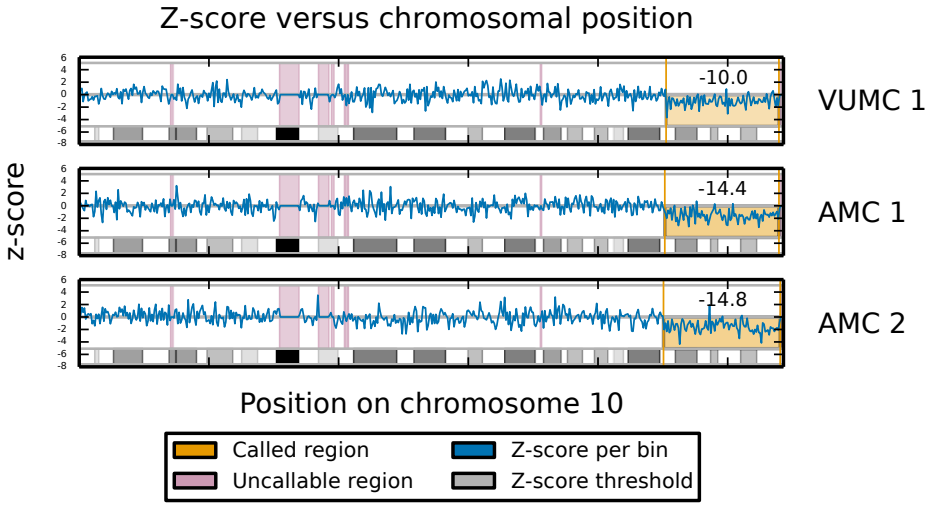
Maternal lymphocytes were grown in RPMI medium, with or without adding 2μM BrdU for 24 hours. Fluorescence in situ hybridization (FISH) was performed with a probe for 10qter (GS-261-B16) and a control probe for region 10pter (GS-23-B11). FISH analysis of approximately 400 interphase nuclei was performed. Array-CGH analysis was performed using the 180K Human CGH oligonucleotide microarray (Agilent Technologies, Santa Clara, CA) with whole genome coverage and an overall median probe spacing of 13 kb according to standard methods.

Results

The original WISECONDOR results of the eight 10(q25→ter) deletions are shown in Figure 1a. The enhanced NIPT pipeline was subsequently used for the three samples that were processed at VUmc (samples VUMC 1, AMC 1 and 2). This high resolution analysis defined the start of the deletions as between bp position 112,750,000 and


Z-score versus chromosomal position
z-score
VUMC 1
AMC 1
AMC 2
EMC 1
EMC 2
EMC 3
RUNMC 1
RUNMC 2
Position on chromosome 10
Z-score windowed method
Z-score individual bin method
Detected deviation by windowed method
Called by windowed method
Detected deviation by individual bin method
Called by individual bin method
Not enough reference bins available
Unmappable region

a.



b.

Figure 1: NIPT results showing a deletion starting in 10q25 in eight pregnancies. a: Initial WISECONDOR output. Data for each sample was analyzed using reference data for the center the sample was processed at. Samples are numbered for each center. b: Plots showing the results of the enhanced WISECONDOR method applied to three samples with the 10q25.2 deletion. The vertical axis shows z-scores for every bin shown on the x-axis. Numbers within the figures show the z-score of the called region. A chromosomal idiogram is visualized at the bottom of each plot. In all samples the deletion starts at 10q25.2, the locus containing the FRA10B site (between 113,001,547 and 113,001,987).

113,250,000 (GRCh37, Figure 1b). Individual start points of the deletions are: VUMC 1 between 113,250,000 and 135,000,000, effect size -3.07%, AMC 1 between 113,000,000 and 135,000,000, effect size -4.54%, AMC 2 between 112,750,000 and 135,250,000, effect size -4.39%. The effect size is the change in read depth compared to the expected amount of reads, as determined by WISECONDOR.

Culture of maternal blood lymphocytes with BrdU to induce a possible FRA10B fragile site, showed indeed a fragile site on one of the chromosomes 10 in 4 out of 4 cases tested (AMC 1, AMC 2, EMC 1 and EMC 2) in up to 60% of the metaphases (Figure 2a). In 30 analyzed metaphases, we did not detect metaphases with an apparent deletion of 10(q25→ter). When cultured according to standard procedures without BrdU, this fragile site was not expressed in blood lymphocytes.

Additional FISH analysis on interphase nuclei of maternal blood lymphocytes cultured with BrdU in 2 cases (AMC 1 and 2), showed a loss of signal for the 10q telomere, but not the 1p telomere in 2-4% of the cells (approximately 400 nuclei, data not shown). These FISH results suggested that carriers of FRA10B may exhibit low-level mosaicism for a 10q terminal deletion. In depth array-CGH analysis of one case (AMC 1) confirmed this finding (Figure 2b). As FRA10B is not associated with any clinical phenotype, we have not tested the carrier status of the fetuses after birth.

Discussion

Aberrations found by NIPT are not always of fetal origin, resulting in false positive NIPT reports. Known examples of confounding factors are confined placental mosaics, maternal CNVs and maternal malignancies. Knowledge of these factors are essential in proper NIPT analysis and counseling. We here describe an additional biological cause for discordant NIPT results. We tested and confirmed FRA10B expansions in 4 mothers where NIPT showed a 10q25 to telomere deletion. FISH and array analysis showed the presence of a maternal low mosaic 10(q25→ter) deletion, probably as a consequence of the expanded fragile site. As approximately 90% of the cell free DNA tested during NIPT is maternal, this low maternal mosaic can be detected during NIPT analysis. Assuming the occurrence rate for FRA10B is 2.5% as stated in previous work, the odds of finding 4 out of 4 individuals with FRA10B at random is ∼3.9e-7, making our observation statistically significant.

A debate is going on whether NIPT should be targeted to trisomy 21, 18 and 13 alone, or that it should be used as a genome wide screening to detect other chromosomal anomalies as well. Although the clear benefit of genome wide testing is that more severe fetal anomalies will be detected [13], one of the arguments against is that it will result in more false positive results and therefore in more invasive follow up tests. However, many of these false positive results can easily be identified and explained without the need for invasive follow up testing. In the case of maternal CNVs causing false positive results, we have argued that using proper bioinformatical tools, it is easy to distinguish maternal CNVs from fetal trisomies [14]. The same is true for maternal malignancies. We here

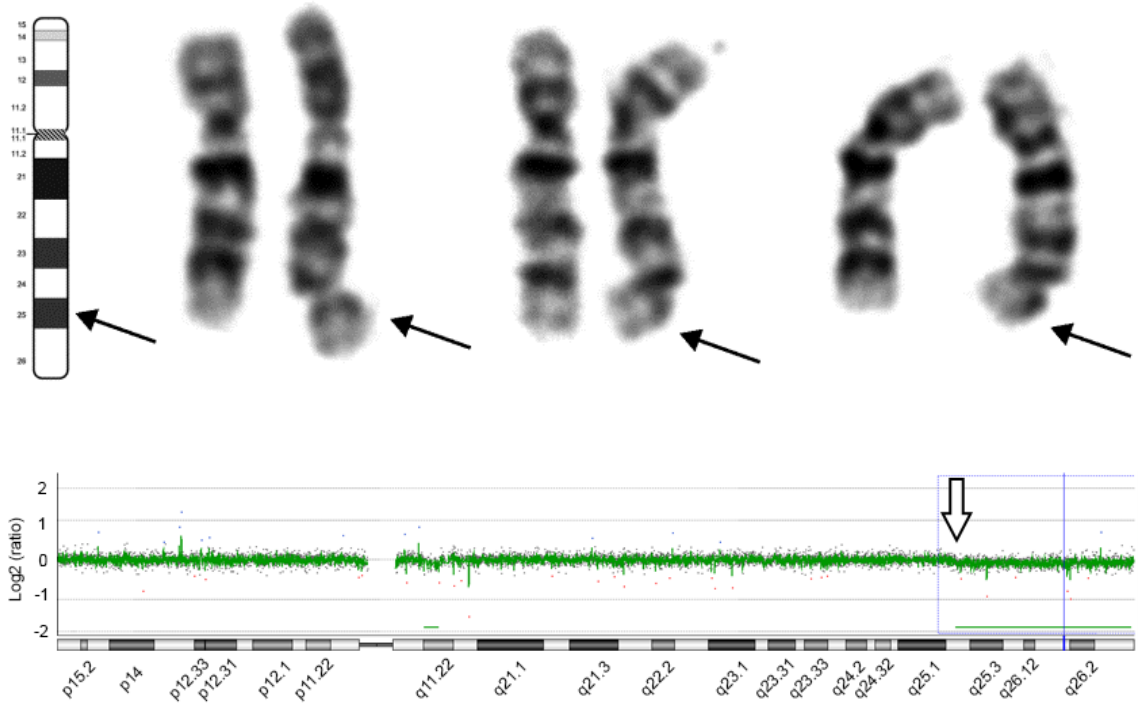


Figure 2: FRA10B expansions and mosaic maternal deletions. a: GTG banding of chromosome 10, after culture of blood lymphocytes in medium with BrdU to induce BrdU-sensitive fragile sites. The fragile site at 10q25 is indicated by an arrow. b: array-CGH analysis of chromosome 10 of one of the mothers shows a low grade mosaic loss of 10q25→qter (breakpoint indicated by an arrow). The log ratio of this deleted region is minus 0.083.

show another example of a relatively common cause of false positive results that does not warrant follow up by invasive testing. If a deletion starting at FRA10B is found it is highly likely that it is caused by a maternal mosaic deletion associated with a repeat expansion at this fragile site. This can be confirmed by maternal testing if preferred, although there is no known phenotype linked to this fragile site, even in homozygous cariers.

Altogether, this finding increases the reliability and health benefits obtained through NIPT. It also proves that classical cytogenetic knowledge is still very important for proper interpretation of NIPT results, as the last scientific papers on FRA10B date from 1998 [8].

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